Study on Resistance of Poplars to *Marssonina brunnea*

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Abstract Inoculation experiment was made for 15 poplar species to determine their resistance to *Marssonina brunnea* by the agar leaf-disc technique and the water-culture shoots technique. The results show that *Populus simonii* and *P. nigra* var. *thevestina* are very susceptible to the disease; *P. davidiana* and *P. koreana* are slightly resistant to the disease; *P. ussuriensis* has high resistance, and the others remain moderate resistance. Experiments are also made for healthy leaves to analysis the inorganic element content and water content. And the results show that the contents of Fe and Ca are remarkbly negatively related to the resistance of poplars to *Marssonina brunnea*.

Key Words: Poplar, *Marssonina* black spot disease, Resistance, Agar leaf-disc technique

Poplar is a kind of growing quickly species with high ornamental and applied values is used extensively in the street of cities botanical garden and farm protection. The poplars are always facing the threats of diseases and insects which can make them decline in growing and even die. One of the important poplar leave diseases is black spot disease caused by *Marssonina brunnea*. This disease makes many poplar seedlings die every year. The disease incidence reached to 60 % and disease index was 30-40 in Qianjiang county of Hubei province during 1981-1982. The disease incidence reached to 99.5% and disease index was 76 in poplar nursery of Kunming City, Yunnan province in 1982. It is a essential question to select resistant species against *Marssonina brunnea* in forestry. We conducted the inoculation experiment for 15 main poplar species in Northeastern area of China by the agar leaf-disc technique, selected the poplar species with high resistance. Meanwhile we conducted the inoculation experiment by water culture shoots as contrast, and determined the poplar healthy leaves inorganic element content and water content in order to find the relation between the poplar resistance and these factors.

Table 1. Disease rating scales and susceptibility classification for poplars resistance by leaf-discs

Disease scales	Disease rating	Lesions per cm² leaf area	Infection levels	Susceptibility scales
I	0	0	Nil	Highly resistant
II	1	1-10	Light	Resistant
III	2	> 10, < 25	Medium	Resistant
IV	3	> 25	Heavy	Susceptible
V	4	> 25	Die	Very susceptible

Materials and Methods

Disease leaves of *Populus simonii* caused by *Marssonina brunnea* were collected in Yuanbaoshan farm, Heilongjiang province in 1989, isolated and cultured in PDA medium, then *Massonina brunnea* produced the conidia which were taken as the inocula in natural light.

15 poplar species were collected from plant garden in Harbin City. They are *Populus pekinensis*, *P. simonii*, P. sp., *P. ussuriensis*, *P. simonii*, *P. pyramidalis*, *P. simonii*, *P. nigra*, *P. stalint*, *P. berolinensis*, *P. russikii*, *P. xiaohei* 14, *P. maximowiczii* CV-2, *P. koreana*, *P. canadensis*, *P. davidiana*, *P. nigra* var. *thevestina*.

Leaf discs (1.0 cm diam.) were punched from mature leaves picked from the leaves growth on poplar shoots, and were inlaid 2 % water agar plates. The conidia of pathogen were diluted into 10^6, 10^5,10^4,10^3 conidium suspension with sterile water. The poplar leaf discs were inoculated with conidium suspension of different concentration by the agar leaf-disc technique, and incubated at 20 ℃ under natural light for 8, 12, 18 days. The infection levels were determined by counting the numbers of lesions/cm² in leaf area (binocular microscope), and assigned to disease scales (Table 1). The

water-culture shoots were inoculated with 10^4 conidium suspension in laboratory and kept in moisture condition for 2 days, then grown in natural environment, and assigned to disease scales (Table 2).

Table 2. Disease rating scales and susceptibility scales for poplar resistance by water-culture shoots

Disease scales	Disease rating	Host reaction scales	Susceptibility scales
I	0	No lesion in leaf	Highly resistant
II	1	Infected leaves less 20%/all inoculated leaves	Resistant
III	2	Infected leaves 21%-50%/all inoculated leaves	Susceptible
IV	3	Infected leaves more than 50%/all inoculated leaves	Very susceptible

The inorganic element contents of poplar healthy leaves were determined by PE-5000 atomic absorption spectrum and the water contents by Ohaus moisture instrument.

Results

Agar leaf-disc water-culture shoots inoculation experiments and the investigation in the fields

The table 3-4 show that *Marssonina brunnea* can infect all species of Tacamahaca section, Aigeiros section and Leuce section, and the all hybrids between Aigeiros section and Tacamahaca section, but the disease levels of every species are different. The *P. simonii*, *P. russikii* of Tacamahaca section, and *P. pekinensis* which is hybrid between Aigeiros section and Tacamahaca section are very susceptible: *P. davidiana* of Leuce section and *P. koreana* of Aigeiros section are resistant; *P. ussuriensis* of Aigeiros section is high resistant; the others are moderate resistant.

Table 3. Disease scales of inoculation 12 d

Poplar species		Disease scales					
		0	1	2	3	4	Disease index
Tacamahaca section	*Populus stalini*	0	9	28	19	0	54
	P. urssikii	0	0	9	39	8	73
	P. canadensis	1	3	13	33	6	68
	P. nigra var. *thevestina*	1	5	5	12	33	82
	P. simonii × *P. nigra*	0	1	12	35	8	71
	P. xiaohei 14	0	5	22	29	0	61
	P. sp	2	9	12	33	0	59
Aigeiros section	*P. simonii*	0	0	1	25	30	88
	P. ussuriensis	0	0	0	0	0	0
	P. berolinensis	0	9	15	31	1	47
	P. koreana	36	13	4	3	0	13
Aigeiros	*P. pekinensis*	0	1	5	43	7	75
	P. simonii × *P. pyramidalis*	0	5	17	25	7	63
Tacamahaca section	*P. maximowiczii* CV-2	1	3	27	24	1	59
Leuce section	*P. davidiana*	34	8	1	5	8	24

Table 4. Disease scales of inoculation 12d by agar leaf-discs

Poplar species		conidia concentration								Trend scales
		10^5		10^4		10^3		10^2		
		Obverse side of leaf	reverse side of leaf	obverse side of leaf	reverse side of leaf	obverse side of leaf	reverse side of leaf	observe side of leaf	reverse side of leaf	
Tacamahaca section	*Populus stalini*	2	2	3	2	2	3	2	2	2
	P. russikii	3	4	3	4	2	3	3	2	3
	P. canadensis	3	3	3	3	3	3	3	3	3
	P. nigra var. *thevestina*	4	4	4	4	4	4	4	2	4
	P. simonii × *P. nigra*	4	3	3	3	2	3	3	3	3
	P. xiaohei14	2	3	2	3	2	3	3	2	3
	P. sp	3	2	2	3	2	3	2	2	3
Aigeiros section	*P. simonii*	4	3	3	4	4	4	4	4	4
	P. ussuriensis	0	0	0	0	0	0	0	0	0
	P. berolinensis	3	3	2	3	2	2	3	2	3
	P. koreana	1	1	1	0	0	0	1	0	1
Aigeios section	*P. pekinensis*	3	3	3	3	3	3	3	3	3
	P. simonii× *P. pyramidalis*	2	3	3	2	2	3	2	2	3
Tacamahaca section	*P. maximowiczii* CV-2	2	3	3	2	2	3	2	2	3
Leuce section	*P. davidiana*	1	1	1	1	1	0	1	0	1

The results show that the resistance of the poplars against pathogen doesn't have relation with the poplar section, but mainly depends on the physiologic and biochemical characteristics of poplar species itself. The disease levels are not different obviously through inoculating to the obverse and the reverse sides of the leaves. The inoculation results of water-culture shoots and investigation in the fields are nearly same with the

results of agar leaf-disc (Table 5-6).

Table 5. The inoculation results by water-culture shoots

Groups	poplar species	No. of inoculation leaves	No. of disease leaves	mean No. of lesions/leaf	Disease incedent (%)
Tacamahaca section	*Populus stalini*	20	6	18	30
	P. russikii	20	15	11	75
	P. canadensis	20	8	15	40
	P. nigra var. *thevestina*	20	16	24	80
	P. simonii × *P. nigra*	20	10	16	50
	P. xiaohei 14	20	8	20	40
	P. sp	20	6	15	30
Aigeios section	*P. simonii*	20	17	25	85
	P. ussuriensis	20	0	0	0
	P. berolinensis	20	6	10	30
	P. koreana	10	1	6	10
Aigeiros section	*P. pekinensis*	20	15	17	75
	P. simonii × *P. pyramidalis*	20	9	17	45
Tacamahaca section	*P. maximowiczii* CV-2	20	7	20	35
Leuce section	*P. davidiana*	20	2	10	10

Table 6. The investigation of poplar resistance in the fields

Group	poplar species	Age of poplar	Planting methods	No. of investigation	Disease incedent?(%)	Disease index
Tacamahaca section	*Populus nigra* var. *thevestina*	1	cutting seedlings	24	91.6	59.7
	P. russikii	1	cutting seedlings	27	88.8	54.3
	P. xiaozhuanica	3	cutting seedlings	61	55.0	35.2
	P. stalini	1	cutting seedlings	13	15.3	5.1
	P. × *canadensis* cv. Robusta	1	cutting seedlings	28	10.7	3.5
	P. canadensis	1	cutting seedlings	24	0	0
Aigeiros section	*P. simonii*	1	cutting seedlings	30	100.0	90.0
	P. simonii	2	stocking seedlings	156	31.4	12.3
	P. pseudo-simonii	1	cutting seedlings	32	100.0	96.8
	P. laurifolia	1	cutting seedlings	9	100.0	81.4
	P. berolinensis	1	cutting seedlings	21	61.9	20.6
	P. ussuriensis	3	stocking seedlings	32	0	0
Aigeiros-Tacamahaca section	*P. simonii* × *P. pyramidalis*	1	cutting seedlings	10	100.0	100.0
	P. simonii × *P. deltoides*	1	cutting seedlings	11	100.0	90.9
	P. pseudo-simonii × *P. pyramidalis*	1	cutting seedlings	10	100.0	86.6
	P. pekinensis	1	cutting seedlings	18	44.4	14.8
	P. simonii × *P. pyramidalis*	1	cutting seedlings	51	100.0	52.9
	P. canadensis CV "1-45"	1	cutting seedlings	26	30.9	9.1
Leuce section	*P. alba* var. *pyramidadlis*	3	sprouting seedlings	55	61.8	49.3

The determining of the inorganic element contents and water contents for poplars

Table 7 shows that the contents of Fe and Ca in the leaves are remarkably negatively related to the resistance of poplars to *Marssonina brunnea*, the higher the contents of Fe and Ca for poplars are, the lower the disease indexes are. But the contents of other elements and water contents are not remarkably related to the resistance of poplars.

Line equation of the relation of Fe element content (x_4) and disease index (y) is as y=96.96-0.18x, and relative coefficient R=-0.72, $F_{0.95}$ (1,12) = 4.75 < F = 12.87; The line equation of the relation of Ca element content (x_6) and disease index (y) is as y=140.09-55.74X6, and relative coefficient R=-0.77, $F_{0.95}$ (1,12)=4.75 < F=17.68.

The contents of Fe and Ca elements of *P. ussuriensis*, *P. koreana* and *P. davidiana* are higher than other poplars, and this three species show high resistance to *Marssonina brunnea*. As we know, Fe and Ca are mainly accumulated in the leaves, Fe may adjust water content in plants, is also the zyme activator, takes part in directly the basic metabolism and N metabolism in the plant body, so it plays a important role in the leaf vitality and the resistance of plants against pathogens. Ca can combine with pectic acid to become pectin acid-Ca, and forms middle glue lamella between the cell and cell, connects one cell with another cell strongly, and resists the pathogen infection, protects the permeable substances in cell from infiltrating.

We sowed seeds of *P. ussuriensis* in three beds in Yuanbaoshan farm, in August, 1989, in order to prove further the resistance of *P. ussuriensis* to *Marssonina brunnea*, per bed was 32 × 1 m^2, the artificial inoculation experiment was conducted (1.4 × 10^4 conidia/ml), the results were negative reaction. We sowed seeds of *P. ussuriensis* and *P. simonii* in beds individually in 1991, and made the artificial inoculation experiment for

two kinds poplars. *P. ussuriensis* still kept negative reaction, but *P. simonii* was infected by *Marssonina brunnea*. The results proved that *P. ussuriensis* was high resistant to *M. brunnea*.

Table 7 Inorganic element contents, water contents and disease index of poplar leaves

Host species	Analytical items								
	Ca (x_1) μg/g	Zn (x_2) μg/g	Mn (x_3) μg/g	Fe (x_4) μg/g	K (x_5) %	Ca (x_6) %	Mg (x_7) %	Water Content (x_8) %	Disease (y)
Populus koreana	11	192.1	42.4	541.7	1.7725	2.0114	0.2303	63.5	13
P. davidiana	11	61.1	154.7	292.5	2.4375	1.9226	0.3010	59.1	24
P. berolinensis	8	73.6	46.3	209.9	2.5275	1.7136	0.2603	64.2	47
P. stalini	9	98.9	23.1	36.4	2.2600	0.9344	0.2980	65.7	54
P. maximowiczii CV-2	8	79.7	28.8	266.8	2.6175	1.8245	0.3393	64.5	59
P. sp.	8	57.5	20.1	216.5	2.1050	1.2575	0.2463	63.6	59
P. simonii × *P. pyramidalis*	10	86.4	25.2	170.2	1.7325	1.4127	0.1525	60.6	63
P. canadensis	6	26.6	40.7	166.7	2.6025	1.2797	0.1550	68.4	68
P. simonii × *P. nigra*	7	95.7	38.4	179.6	1.9225	1.4602	0.2708	60.9	71
P. russikii	8	66.5	43.3	158.0	2.1125	1.1656	0.3178	66.1	73
P. pekinensis	3	51.6	25.8	192.5	1.8480	1.2702	0.2148	64.3	75
P. nigra var. *thevestina*	10	154.8	46.6	255.0	3.1700	1.4159	0.4030	77.8	82
P. simonii	8	183.0	31.6	148.5	1.7175	1.3937	0.2558	55.7	88
P. ussuriensis	9	99.8	45.5	317.7	2.4360	2.2046	0.5023	67.3	0

Conclusions and Discussions

We selected *P. ussuriensis* as high resistant. *P. koreana* and *P. davidiana* are resistant, *P. simonii* and *P. nigra* var. *thevestina* are very susceptible. *P. simonii* × *P. nigra*, *P. pekinensis* and *P. russikii* are susceptible, the others are moderate.

Fe and Ca element content of poplar leaves is negatively related to resistance of poplar to disease indexes, this is the first time to prove the relation between poplar resistance and inorganic element content, and provides also the reference to study the resistant mechanism of the other leaf diseases.

Agar leaf-disc techniques is very simple and the inocula are united in amounts, the environment conditions can be controlled by people, experiment materials can get easily, the experiments can be conducted repeatedly, so this method can be used in studying other leaf disease.

We suggest that the concentration of 1000 conidia/disc should be the common inoculation concentration in laboratory because this concentration can make the poplars remarkably showing resistance to the pathogen.

Marssonina brunnea can infect the poplar leaves through obverse sides and reverse sides of leaves, and no remarkable difference between both sides of leaves, this shows that the pathogen infects the poplar through the epidermis, and also enter into the host bodies through leaves stomas.

The inoculation results show that *Marssonina brunnea* can infect widely Tacamahaca section, Leuce section, Aigeiros section and hybrids of Aigeiros × Tacamahaca section. The differences of poplar resistance to the pathogen throws the limits among the poplar sections into confusion. For example *Populus ussuriensis* is high resistant to *Marssonina brunnea*, this result turns out. Contrary to the concept of the Aigeiros section being susceptible to the pathogen for long time in plant pathology, so it needs to further discuss and study if the limits among the poplar section are related with host resistance.

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